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ORIGINAL ARTICLE

Genetic analysis of net form net blotch resistance in barley lines CIho 5791 and Tifang against a global collection of *P. teres* f. *teres* isolates

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Abstract

Key message **A CIho 5791 × Tifang recombinant inbred mapping population was developed and used to identify major dominant resistance genes on barley chromosomes 6H and 3H in CI5791 and on 3H in Tifang.**

Abstract The barley line CIho 5791 confers high levels of resistance to *Pyrenophora teres* f. *teres*, causal agent of net form net blotch (NFNB), with few documented isolates overcoming this resistance. Tifang barley also harbors resistance to *P. teres* f. *teres* which was previously shown to localize to barley chromosome 3H. A CIho 5791 × Tifang F_6 recombinant inbred line (RIL) population was developed using single seed descent. The Illumina iSelect SNP platform was used to identify 2562 single nucleotide polymorphism (SNP) markers across the barley genome, resulting in seven linkage maps, one for each barley chromosome. The CIho 5791 × Tifang RIL population was evaluated for NFNB resistance using nine *P. teres* f. *teres* isolates collected globally. Tifang was resistant to four of the isolates tested whereas CIho 5791 was highly resistant to all nine isolates. QTL analysis indicated that the CIho 5791 resistance mapped to chromosome 6H whereas the Tifang resistance mapped to chromosome 3H. Additionally, CIho 5791 also harbored resistance to two Japanese isolates that mapped to a 3H region similar to that of Tifang. SNP markers and RILs harboring both 3H and 6H resistance will be useful in resistance breeding against NFNB.

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Introduction

Net blotch, caused by *Pyrenophora teres*, is present in most barley production regions of the world, including the Middle East, Australia, Asia, Europe, Africa, and South and North America (Mathre 1997). The pathogen is most prevalent where barley is planted under cool wet conditions; however, it also exists in warm dry areas (Shipton et al. 1973). *P. teres* exists in two forms, *P. teres* f. *teres* and *P. teres* f. *maculata*, causing net form net blotch (NFNB) and spot form net blotch (SFNB), respectively. The NFNB disease is initially observed as small circular and elliptical dot-like lesions that soon develop into dark brown blotches containing longitudinal and transverse striations forming a net-like pattern (Steffenson and Webster 1992; Mathre 1997). For highly resistant barley lines, dot-like lesions do not develop into the net-like pattern, but remain restricted.

Geschele (1928) showed that resistance to NFNB was inherited in a Mendelian fashion (Reviewed in Liu et al. 2011). Later, three incompletely dominant resistance genes reported as Pt_1, Pt_2 and Pt_3 were shown to be effective against *P. teres* isolates collected in California (Mode and Schaller 1958; Schaller 1955) and several other breeding lines were reported to harbor single dominant resistant genes (Gray 1966; McDonald and Buchannon 1962). Khan and Boyd (1969a, b) were the first to report the physiological specialization of the pathogen which was useful in the evaluation of sources of resistance that correlated with

differences in virulence. NFNB resistance genes *Rpt1a*, *Rpt3d*, *Rpt1b* and *Rpt2c* were identified by trisomic analysis on barley chromosomes 3H, 2H, 3H and 5H, respectively (Bockelman et al. 1977). As is often the case, inheritance of resistance in adult plants under field conditions was shown to be more complex as compared to seedling resistance (Arabi et al. 1990; Douglas and Gordon 1985; Steffenson and Webster 1992). Dominant susceptibility genes have also been identified in seedlings (Ho et al. 1996; Abu Qamar et al. 2008; Liu et al. 2015), as well as the potential for the corresponding pathogen effectors (Liu et al. 2015; Shjerve et al. 2014), showing the complexity of this host-pathogen interaction.

In several barley backgrounds, resistance to NFNB has mapped to chromosome 6H (reviewed in Liu et al. 2011) but other studies have shown that a similar region on chromosome 6H consists of genes that confer dominant susceptibility to different pathotypes of *P. teres* f. *teres*. (Abu Qamar et al. 2008; Liu et al. 2011, 2015; Shjerve et al. 2014; Richards et al. 2016). Several studies have been performed using differential sets of barley lines that exhibited different resistance patterns when inoculated with NFNB isolates collected from different parts of the world (Steffenson and Webster 1992; Wu et al. 2003; Gupta and Loughman 2001; Cromey and Parkes 2003; Jalli 2004; Tekauz 1990; Jonsson et al. 1997; Khan and Boyd 1969b; Liu et al. 2011; Jalli and Robinson 2000). These studies indicated the presence of several different avirulence and/or virulence factors that theoretically correspond to different resistance/susceptibility genes in these barley lines.

CIho 5791 is an Ethiopian breeding line reported to show high levels of resistance against *P. teres* f. *teres* isolates (Mode and Schaller 1958; Khan and Boyd 1969a, b, 1971; Tekauz 1990; Steffenson and Webster 1992; Wu et al. 2003; Cromey and Parkes 2003; Jalli 2004) and Tifang is a Manchurian line reported to also show resistance against some *P. teres* f. *teres* isolates (Steffenson and Webster 1992; Wu et al. 2003; Cromey and Parkes 2003; Jalli 2004; Jonsson et al. 1997; Khan and Boyd 1969b, 1971; Jalli and Robinson 2000). Using trisomic analysis, Bockelman et al. (1977) reported that Tifang had resistance located on chromosome 3H (Bockelman et al. 1977). Based on the phenotypic differences observed in the resistance patterns against various *P. teres* f. *teres* isolates, CIho 5791 and Tifang were selected to develop a population and study the genetics of resistance found in each line.

Materials and methods

Biological materials

One hundred and seventeen $F_{2:6}$ recombinant inbred lines (RILs) were developed by single seed descent from a cross between CIho 5791 (hereafter referred to as CI5791) and Tifang resulting in a CI5791 (female) × Tifang RIL population (hereafter referred to as the CT population). F_2 individuals were similarly derived from crosses of CI5791 and Tifang to be used in evaluating gene action. The CT population, F_2 individuals, and the parents were evaluated for reactions to nine *P. teres* f. *teres* isolates that had diverse geographic origins, including LDNH04Ptt-19, Tra-A5, FGOH04Ptt-21, 15A, 6A, JPT0101, JPT9901, Br. Pteres and BB06 (Table 1). This is the most geographically diverse set of isolates that we have and several of these isolates have been used in other studies including as parents of mapping populations.

Table 1 Source and collection location information for each of the nine *P. teres* f. *teres* isolates used in this study

Isolate	Location	References	Source
LDNH04Ptt-19	North Dakota, USA	–	Tim Friesen
Tra-A5	Montana, USA	–	Tim Friesen
FGOH04Ptt-21	North Dakota, USA	–	Tim Friesen
JPT0101	Japan	–	Jack Rasmussen
JPT9901	Japan	(Liu et al. 2015)	Jack Rasmussen
15A	California, USA	(Steffenson and Webster 1992; Wu et al. 2003; Shjerve et al. 2014; Liu et al. 2015)	Brian Steffenson
6A	California, USA	(Steffenson and Webster 1992; Wu et al. 2003; Shjerve et al. 2014; Liu et al. 2015)	Brian Steffenson
Br.Pteres	Brazil	(Liu et al. 2015)	Flavio Santana
BB06	Denmark	(Liu et al. 2015)	Lise Nistrup Jorgensen

References for each isolate are included if available

Genotypic analysis

DNA was extracted from the CT population and parents using the Qiagen Biosprint 15 Plant Extraction kit (Shjerve et al. 2014). After obtaining DNA, the Illumina iSelect SNP platform (Comadran et al. 2012), including 7824 SNP markers, was used to genotype the population. The Infinium SNP assay was performed following the manufacturer's instructions (Illumina 2010). Genotype calling was done using the genotyping module implemented in the GenomeStudio software v.2011.1 developed by Illumina (San Diego, CA). Genotype calls were then manually inspected for call accuracy.

Map construction

The Microsoft Excel-based software program MapDisto version 1.7.5 (Lorieux 2012) was used to construct the genetic linkage maps. The 'find groups' command was used to identify linkage groups with $LOD_{min} = 3.0$ and $r_{max} = 0.3$. The 'order sequence' command was used to establish the initial order of markers in each linkage group. The 'ripple order', 'check inversions', and 'drop locus' commands were used to refine and validate the final order of the markers. The 'draw all sequences' command was used to obtain a graphical representation of the maps for all the linkage groups. For QTL analysis, co-segregating markers were identified from the genetic maps, and a single marker within each set of co-segregating markers was retained while the remaining redundant markers were removed. Preference for the marker to be retained at each locus was given to the one with the least amount of missing data. The maps were then reconstructed in MapDisto and the data exported for QTL analysis using the computer program QGene v4.3 (Joehanes and Nelson 2008).

Genetic map comparison

Population sequencing (POPSEQ) positions (Mascher et al. 2013) of the Illumina iSelect SNPs previously described by Cantalapiedra et al. (2015) in the tool BARLEYMAP were utilized to determine map concordance. Data was imported into Microsoft Excel and the command 'vlookup' was executed to create a cross reference file containing all available POPSEQ positions of the markers utilized in the genetic map construction. If no POPSEQ position was available from this dataset for markers flanking a QTL, BLAST searches of the barley genome were conducted (http://webblast.ipk-gatersleben.de/barley/viroblast.php).

Phenotypic analysis

All *P. teres* f.*teres* isolates were grown on V8-PDA (150 ml V-8 juice, 10 g Difco PDA, 3 g $CaCO_3$, 10 g agar, and 850 ml distilled water). Petri plates were kept in a dark cabinet at room temperature for 5 days, followed by 24 h of light at room temperature, followed by 24 h of dark at 15 °C. Plates were then flooded with distilled water and conidia were harvested from plates using an inoculating loop. The inoculum was collected and diluted with distilled water to obtain 2000 spores/ml. One drop of Tween 20 was added to every 50 ml of inoculum to reduce spore clumping (Abu Qamar et al. 2008).

Individual RILs were planted along with the parents in a rack containing 98 cone-tainers (Stuwe and Sons, Inc., Corvallis, OR) with 'Tradition' barley planted as the border to reduce any edge effect. Inoculations were done as described by Friesen et al. (2006). When the secondary leaves were fully expanded, plants were inoculated with a conidial solution of individual *P. teres* f. *teres* isolates using an air sprayer (Huskey, model# HDS790) until a heavy mist covered all the leaves before runoff occurred. After inoculations, plants were placed in 100 % relative humidity in the light at 21 °C for 24 h and then placed in a growth chamber under a 12 h photoperiod at 21 °C. Disease reactions were evaluated seven post-inoculation because, for this fungus, under these conditions, a 7 day evaluation was found to be optimal. These reactions were evaluated on a 1 to 10 scale as described by Tekauz (1985) where reaction type 1 was the most resistant and reaction type 10 was the most susceptible. Greater than or equal to 4 were considered as the susceptible rather than 5 as suggested by Tekauz (1985). Three un-randomized replicates with borders were completed for each isolate across the whole population (Online Resource 1). For F_2 analysis, F_2 individuals were planted in a single cone-tainer and inoculated with the nine *P. teres* f. *teres* isolates separately, similar to the RIL population. Each plant was evaluated individually for disease reaction to each isolate.

QTL analysis

The average of three replicates and the MapDisto marker data were exported to QGene software v 4.3.0 for QTL analysis (Joehanes and Nelson 2008). The critical logarithm of the odds ratio (LOD) threshold for each data set was calculated by performing 1000 permutations and the obtained value at the $\alpha_{0.01}$ level was used as the critical LOD threshold. Composite interval mapping (CIM) was performed by selecting the LOD value as the test statistic. QTL analysis was carried out by selecting a particular trait and looking across all the linkage groups for the significant QTL. The cofactor parameter was selected as a default parameter to identify the most significant marker underlying each QTL. The chromosome display command was used to view the marker loci on each linkage group.

Statistical analysis

Least significant differences (LSD) were identified to determine separation between the average phenotypic reactions for genotypic classes identified in the CT population. SAS 9.4 (SAS Institute Inc., 2013) was used to perform the LSD tests at $\alpha = 0.05$.

Results

Linkage mapping

The Barley iSelect chip used for marker identification featured 7824 SNP markers distributed across the barley genome. 2562 of the 7824 SNP markers were polymorphic in our CT population and were, therefore, used for linkage mapping analysis. The markers were assembled into seven linkage groups corresponding to the seven barley chromosomes (Online Resource 2). The linkage groups spanned a total genetic distance of 1012.2 cM, with the chromosome 6H linkage group being the shortest (113.6 cM) and the chromosome 5H linkage group being the longest (184.6 cM) (Table 2). The number of SNP markers per chromosome ranged from 243 (chromosome 1H) to 503 (chromosome 5H). A total of 827 unique loci were detected by the 2562 SNP markers yielding an average density of 1.2 cM/locus. 19 gaps were identified on the linkage groups with sizes ranging from 5 to 10.8 cM and these gaps were located at different positions on the linkage groups. One marker from each of the 827 loci was chosen to derive a non-redundant marker set for subsequent QTL analysis.

Genetic map comparison

A total of 2562 markers that were identified as polymorphic between CI5791 and Tifang were compared to the barley genome via data from BARLEYMAP to obtain POPSEQ genetic positions. Of the 2562 markers, a POPSEQ locus was obtained for 1938 markers and used for collinear comparison to the barley genome (Online Resource 2). POPSEQ chromosomal anchoring of the 1938 markers nearly perfectly correlated with the CI5791 × Tifang linkage groups, with the exception of marker SCRI_RS_180004, which was anchored to chromosome 7H via POPSEQ and to chromosome 6H in the CI5791 × Tifang population. However, upon further examination via BLAST searches of the barley genome, the second best BLAST hit (87 % identity) for marker SCRI_RS_180004 was on chromosome 6H at 54.88 cM. Markers that flank SCRI_RS_180004 in the CI5791 × Tifang linkage map have POPSEQ positions ~55 cM on chromosome 6H, indicating that this marker may have been non-specific in this population. Scatterplots were constructed to compare the genetic positions of the remaining markers (Online Resource 3). General collinearity was observed, with only a few minor discrepancies between the CI5791 × Tifang and POPSEQ genetic positions.

Phenotypic analysis

Homogeneity between replicates was high with Pearson's correlation coefficients between replicates ranging from 0.6 to 0.9 (Online Resource 4), therefore, reps were averaged and used for analysis. CI5791 was highly resistant to all nine isolates (average disease reactions of less than 2.0) and Tifang was resistant to four of the nine isolates including 15A, 6A, Br. Pteres and BB06 (average disease reactions of less than 2) (Table 3; Fig. 1). LDNH04Ptt-19, Tra-A5, FGOH04Ptt-21 and JPT9901 were virulent on Tifang with average disease reactions equal to or greater than 5.0. JPT0101 was more virulent on Tifang compared to CI5791, with average disease reaction types of 4.00 and 1.0, respectively, indicating a relatively lower level of virulence on Tifang for this isolate compared to the other virulent isolates (Table 3; Fig. 1).

Across the CT population, similarities also arose among the members of three groups, i.e., the Northern Great Plains isolates (LDNH04Ptt-19, Tra-A5, FGOH04Ptt-21), Japanese isolates (JPT0101 and JPT9901) and a geographically diverse group of isolates consisting of two California isolates (15A and 6A), a Brazilian isolate (Br. Pteres) and a

Table 2 Summary of the seven linkage maps developed in the CT RIL population

Chromosome	SNP markers	No. unique loci	Length (cM)	Marker density (cM/locus)
1H	243	90	133.5	1.5
2H	480	143	161.2	1.1
3H	407	122	156.7	1.3
4H	250	91	115.3	1.3
5H	503	146	184.6	1.3
6H	323	103	113.6	1.1
7H	356	132	147.3	1.1
Total	2562	827	1012.2	1.2

Table 3 Disease reaction types of parents and genotypic classes of the CT RIL population

Isolate[a]	LSD[b]	CI5791[c]	Tifang[c]	$6H_{CI5791}$/ $3H^{d}_{Tifang}$	$6H_{CI5791}$/ $3H^{d}_{CI5791}$	$6H_{Tifang}$/ $3H^{d}_{Tifang}$	$6H_{Tifang}$/ $3H^{d}_{CI\ 5791}$
LDNH04Ptt-19	0.4220	1.00	7.17	1.12 A	1.10 A	5.99 B	6.21 B
Tra-A5	0.4897	1.00	7.33	1.25 A	1.10 A	6.47 B	6.82 B
FGOH04Ptt-21	0.4386	1.25	5.50	1.80 A	2.04 A	5.97 B	6.98 C
JPT0101	0.4572	1.00	4.00	1.13 A	1.01 A	4.85 C	1.77 B
JPT9901	0.4868	1.17	6.17	1.26 A	1.11 A	6.54 C	3.96 B
15A	0.5653	1.00	1.50	1.08 A	1.04 A	2.32 B	6.14 C
6A	0.6423	1.00	1.75	1.09 A	1.69 AB	1.89 B	4.94 C
Br. Pteres	0.3692	1.17	1.00	1.03 A	1.35 A	1.28 A	3.12 B
BB06	0.3592	1.00	1.17	1.00 A	1.12 A	1.16 A	3.35 B

[a] The nine *P. teres* f. *teres* isolates used in the analysis, which include the Northern Great Plains isolates (LDNH04Ptt-19, Tra-A5, FGOH04Ptt-21), the Japanese isolates (JPT0101 and JPT9901), the California isolates (15A and 6A), the Brazilian isolate (Br. Pteres) and the Danish isolate (BB06)

[b] Least significant difference (LSD) calculated at $P = 0.05$ for each of the isolates

[c] Parents, CI5791 and Tifang, used to develop the CT mapping population

[d] The genotypic classes for the RIL population based on the presence of the most significant marker at the 3H and 6H resistance loci

Danish isolate (BB06). R:S segregation ratios of the three Northern Great Plains isolates that were virulent on Tifang (LDNH04Ptt-19, Tra-A5 and FGOH04Ptt-21) were not significantly different from a 1:1 when using a reaction type of 4.0 as the susceptible cutoff, indicating a single major gene conferring resistance or susceptibility (Table 4). For the Japanese isolates (JPT0101 and JPT9901) the R:S ratio was narrowly but still significantly different from a 3:1 ratio ($\chi^2 = 3.9$; $P = 0.048$ and $\chi^2 = 4.1$; $P = 0.043$, respectively) (Table 4).

Among isolates with avirulent phenotypes on both parents, inoculation of the California isolates (15A and 6A) resulted in a R:S segregation ratio that was not significantly different from a 3:1, indicating the presence of two resistance genes but with one coming from each parent. When using the same resistant/susceptible cutoff as we did for the other isolates, the Brazilian (Br. Pteres) and the Danish (BB06) isolates showed R:S ratios that were significantly different from a 3:1 ratio ($\chi^2 = 19.132$; $P < 0.0001$ and $\chi^2 = 17.220$; $P < 0.0001$) indicating the potential of at least one resistance gene coming from each parent but with additional genes resulting in a complex quantitative inheritance (Table 4).

F_2 analysis

To evaluate the resistance gene action conferred by both CI5791 and Tifang, F_2 analysis was performed using ≥4 as the susceptible cut off. CI5791 × Tifang F_2 individuals showed a 3:1 (R:S) ratio when inoculated with LDNH04Ptt-19, Tra-A5 and FGOH04Ptt-21 isolates (Table 4; Fig. 2), confirming the single resistance gene interpretation from the RIL population. For the isolates avirulent on both parental lines, including the California isolates (15A and 6A), the Brazilian isolate (Br. Pteres) and the Danish isolate (BB06), the F_2 individuals showed a R:S ratio not significantly different from 15:1 (Table 4; Fig. 2) indicating two dominant resistance genes. The Japanese isolates (JPT0101 and JPT9901) also showed a R:S ratio not significantly different from a 15:1 (Table 4; Fig. 2) indicating the presence of two dominant resistance genes, both coming from CI5791, matching the results from the RIL population.

QTL analysis

By performing 1000 permutations on each data set, LOD value thresholds ($P = 0.01$) were obtained that ranged from 3.6 to 4.0. Hence, the highest stringency identified (4.0) was used as a critical LOD threshold value for identifying significant QTL.

A major resistance QTL located on chromosome 6H with resistance effects contributed by CI5791 was identified for all nine isolates tested (Table 5; Fig. 3). The three Northern Great Plains isolates, LDNH04Ptt-19, Tra-A5 and FGOH04Ptt-21 showed only the 6H QTL (Table 5; Fig. 3). However, for all isolates avirulent on both parents, including 15A, 6A, Br. Pteres, and BB06, a 3H resistance QTL was identified with resistance effects contributed by Tifang (Table 5; Fig. 3). A similarly located chromosome 3H QTL was also identified for JPT0101 and JPT9901 (Fig. 3), however, the 3H resistance to these two Japanese isolates was conferred by CI5791 (Table 5; Fig. 3). Additional relatively minor 1H (Online Resource 5) and 3H QTL were also observed for 6A with a LOD value of 5.4 and 5.0, respectively.

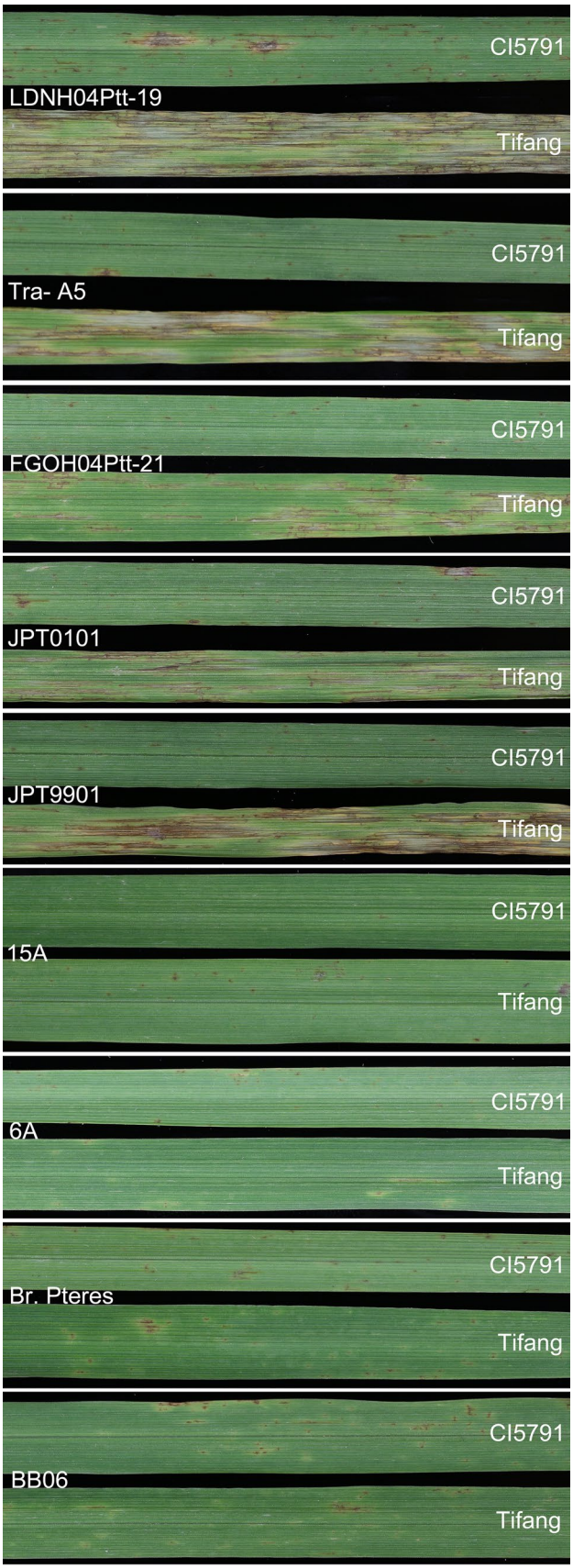

Fig. 1 Disease reactions on CI5791 and Tifang for each of the nine ▶ *P. teres* f. *teres* isolates. Barley parental lines are indicated on the right and *P. teres* f. *teres* isolates are indicated on the left. All tested isolates were avirulent on CI5791. 15A, 6A, Br. Pteres, and BB06 isolates were avirulent on both CI5791 and Tifang. The Northern Great Plains isolates (LDNH04Ptt-19, Tra-A5 and FGOH04Ptt-21) and the Japanese isolates (JPT0101 and JPT9901) were virulent on Tifang

The most significant markers at the major 3H and 6H QTL regions (i.e., SCRI_RS_140091 for 6H and SCRI_RS_221644 for 3H) were used to create four genotypic classes (Table 3). The genotypic classes consisted of $6H_{CI5791}/3H_{CI5791}$, $6H_{CI5791}/3H_{Tifang}$, $6H_{Tifang}/3H_{CI5791}$, and $6H_{Tifang}/3H_{Tifang}$ (Table 3) and were used to evaluate the data sets for each of the nine *P. teres* f. *teres* isolates. The Northern Great Plains isolates LDNH04Ptt-19, Tra-A5 and FGOH04Ptt-21 showed a QTL on chromosome 6H alone that was conferred by CI5791. The genotypic classes containing the $6H_{CI5791}$ marker type (i.e. $6H_{CI5791}/3H_{CI5791}$, $6H_{CI5791}/3H_{Tifang}$) were highly resistant regardless of the 3H genotype with the $6H_{CI5791}/3H_{CI5791}$ and the $6H_{CI5791}/3H_{Tifang}$ genotypes having disease reaction types ranging from 1.10 to 2.04 and 1.12 to 1.80, respectively, and the $6H_{Tifang}/3H_{Tifang}$ and $6H_{Tifang}/3H_{CI5791}$ genotypic classes showing phenotypic reactions ranging from 5.99 to 6.47 and 6.21 to 6.98, respectively (Table 3). Interestingly, for the FGOH04Ptt-21 data set, there was also a significant difference between the $6H_{Tifang}/3H_{CI5791}$ and $6H_{Tifang}/3H_{Tifang}$ genotypes, although, based on the QTL analysis, this is not explained by the 3H locus (Fig. 3).

For the Californian, Brazilian, and Danish isolates, the 3H resistance conferred by Tifang and the 6H resistance conferred by CI5791 were both highly effective. As observed for the Northern Great Plains isolates, the presence of the CI5791 type marker at the 6H locus ($6H_{CI5791}/3H_{CI5791}$ and $6H_{CI5791}/3H_{Tifang}$) showed complete resistance with reaction types ranging from 1.04 to 1.69 and 1.00 to 1.09, respectively (Table 3). When Tifang alleles were present at both 3H and 6H ($6H_{Tifang}/3H_{Tifang}$) the reaction types ranged from 1.16 to 2.32 showing the effectiveness of the 3H resistance being conferred by Tifang (Table 3) even in the absence of the 6H CI5791 resistance. When the Tifang allele at the 6H locus was combined with the CI5791 allele at the 3H locus ($6H_{Tifang}/3H_{CI5791}$), moderately susceptible to susceptible reactions were observed ranging from 3.12 to 6.14. For BB06 and Br. Pteres, no significant differences in resistance were identified between genotypes harboring Tifang alleles ($6H_{Tifang}/3H_{Tifang}$) and those harboring CI5791 alleles at both loci ($6H_{CI5791}/3H_{CI5791}$), however, the California isolate 15A showed a significant difference between

Table 4 Resistant:susceptible (R:S) segregation ratios of CI5791 × Tifang RIL population and F_2 individuals

Isolates	Observed R:S ratio[e] (RILs)	Expected ratio	R:S ratio[e] (F_2)	Ratio
LDNH04Ptt-19	63:54	1:1	40:9	3:1
Tra-A5	63:51	1:1	35:15	3:1
FGOH04Ptt-21	59:58	1:1	31:14	3:1
JPT0101	97:20	3:1[a]	44:3	15:1
JPT9901	74:37	3:1[b]	51:3	15:1
15A	81:36	3:1	52:4	15:1
6A	92:24	3:1	48:2	15:1
Br. Pteres	99:7	3:1[c]	35:2	15:1
BB06	98:8	3:1[d]	43:4	15:1

[a] Significantly different from 3:1($P = 0.048$)

[b] Significantly different from 3:1 ($P = 0.043$)

[c] Significantly different from 3:1 ($P < 0.0001$)

[d] Significantly different from 3:1 ($P < 0.0001$)

[e] R:S ratios are based on a ≥4.0 cutoff for susceptibility

these two genotypic groups, indicating that although both $6H_{CI5791}$ and $3H_{Tifang}$ confer resistance, the 6H resistance conferred by CI5791 is significantly more effective, at least to the California isolate 15A.

For the Japanese isolates, resistance was conferred by CI5791 only, with Tifang being significantly more susceptible (Table 3). Interestingly, the presence of the CI5791 allele at either the 3H or 6H locus (i.e. $6H_{CI5791}/3H_{CI5791}$ and $6H_{Tifang}/3H_{CI5791}$ and $6H_{CI5791}/3H_{Tifang}$) conferred a resistant reaction indicating that in addition to the CI5791 6H resistance, CI5791 harbours an isolate-specific resistance at a similar position on chromosome 3H as that of Tifang. Genotypes having the CI5791 6H allele were highly resistant as with the other isolates ranging from 1.01 to 1.11 for the $6H_{CI5791}/3H_{CI5791}$ genotype and 1.13 to 1.26 for the $6H_{CI5791}/3H_{Tifang}$ genotype. Unlike the California/Brazil/Denmark group, the $6H_{Tifang}/3H_{CI5791}$ was significantly more resistant, ranging from 1.77 to 3.96 compared to the genotypic group containing Tifang alleles at both loci ($6H_{Tifang}/3H_{Tifang}$), which showed moderately susceptible to susceptible reactions ranging from 4.85 to 6.54. Additionally, as was seen with the California isolates, genotypes harboring the CI5791 6H resistance alone showed a significantly more resistant reaction than the genotypes harboring the CI5791 3H resistance (Table 3).

Discussion

Several previous studies revealed the presence of both resistance and susceptibility genes at the centromeric region of barley chromosome 6H (reviewed in Liu et al. 2011). Here we showed that NFNB resistance conferred by CI5791 was effective against a global collection of *P. teres* f. *teres* and this resistance also mapped to a similar centromeric region on barley chromosome 6H. Unlike several of the other 6H studies, the 6H resistance conferred by CI5791 was highly effective with almost no disease-associated damage to the leaf, outside of a pinpoint dark brown lesion (Fig. 1). Based on phenotypic analysis of F_2 individuals and phenotypic and QTL analysis of an RIL population, it was clear that the 6H resistance conferred by CI5791 was dominant.

When dividing the population into genotypic classes that did or did not have the CI5791 6H alleles, the lines with CI5791 alleles ranged in disease reaction from 1.0 to 2.0 which are highly resistant reactions on the 1 to 10 Tekauz (1985) scale. The complete effectiveness of the CI5791 resistance to all the isolates tested indicates the potential usefulness and durability of this gene..

NFNB resistance/susceptibility loci have also been identified on chromosome 3H (Graner et al. 1996; Raman et al. 2003; Gupta et al. 2004; Yun et al. 2005), including NFNB studies on Tifang (Schaller 1955) where resistance was located to chromosome 3H using trisomic analysis (Bockelman et al. 1977). However, no chromosome 3H map location was identified. Our current study mapped and genetically characterized the 3H locus and showed that resistance coming from chromosome 3H was effective against six of the nine isolates used in this study. Interestingly, 3H resistance effective against the Danish, the Brazilian, and the two California isolates was conferred by Tifang alleles, but the 3H resistance effective against the two Japanese isolates was conferred by the CI5791 alleles, indicating the presence of allelic variation of a single resistance gene or two linked resistance genes, one in CI5791 and one in Tifang.

RIL population analysis did not clearly define resistance gene action for the Japanese, Brazilian or Danish isolates (Table 4). However, the F_2 results did indicate that the 3H resistance conferred by both Tifang and CI5791 as well as the 6H resistance conferred by CI5791 were dominant. It is possible that, similar to the 6H centromeric region identified here and by others, the 3H locus is also a complex region harboring different alleles of the same gene in Tifang and CI5791 or at least two closely linked resistance genes conferring resistance to different pathotypes. The generation of a larger population, fine mapping, and gene cloning will be necessary to characterize these regions.

Recently, several necrotrophic specialist pathogens have been shown to produce necrotrophic effectors (NEs) that are effective at triggering the host programmed cell death (PCD) response to induce necrosis for the purpose of extracting nutrients from the host (Liu et al. 2012; Lorang et al. 2012; Ciuffetti et al. 2010). *P. teres* f. *teres* has also

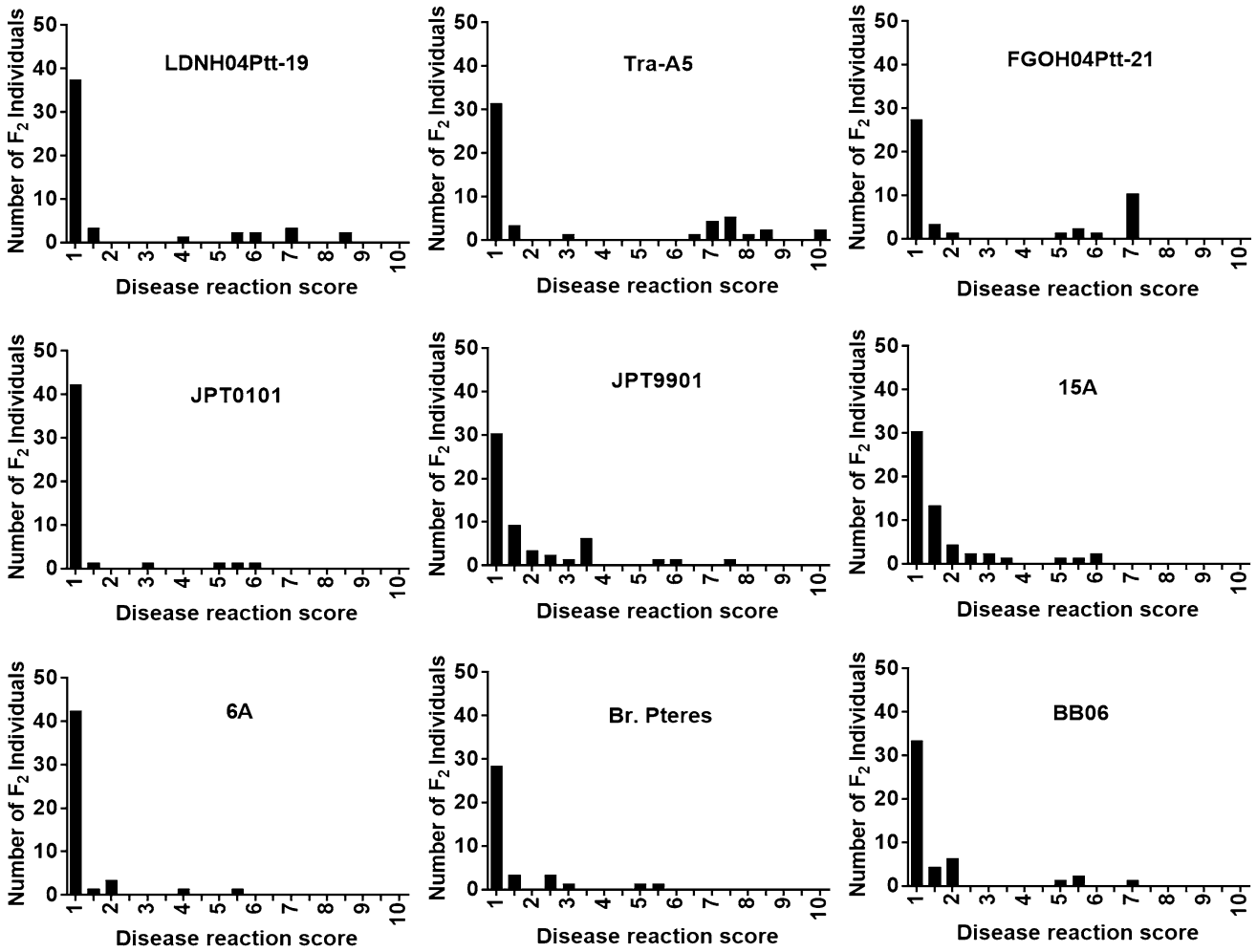

Fig. 2 Histograms showing the phenotypic reactions obtained for F_2 individuals of the CI5791 × Tifang cross inoculated with the nine *P. teres* f. *teres* isolates (LDNH04Ptt-19, Tra-A5, FGOH04Ptt-21, JPT0101, JPT9901, 15A, 6A, Br. Pteres and BB06). The *y*-axis shows the number of the F_2 individuals and the *x*-axis shows the disease reaction score categories separated in 0.5 point intervals

Table 5 Major quantitative trait loci associated with resistance to barley net form net blotch caused by *P. teres* f. *teres* isolates in the CT RIL population

Isolates	LOD values (percent variation explained)			
	3H	Resistance source	6H	Resistance source
LDNH04-Ptt-19	–	–	48.0 (83.0 %)	CI5791
Tra-A5	–	–	47.0 (86.0 %)	CI5791
FGOH04Ptt-21	–	–	35.0 (73.0 %)	CI5791
JPT0101	11.0 (23.0 %)	CI5791	18.0 (37.0 %)	CI5791
JPT9901	6.2 (8.1 %)	CI5791	29.0 (63.0 %)	CI5791
15A	13.0 (18.0 %)	Tifang	21.0 (45.0 %)	CI5791
6A[a]	16.0 (23.0 %)	Tifang	19.0 (30.0 %)	CI5791
Br. Pteres	16.0 (28.0 %)	Tifang	9.9 (25.0 %)	CI5791
BB06	16.0 (26.0 %)	Tifang	11.0 (26.0 %)	CI5791

[a] Isolate 6A had additional QTL peaks on chromosomes 1H and 3H with LOD scores of 5.4 (11.0 %) and 5.0 (8.0 %), respectively

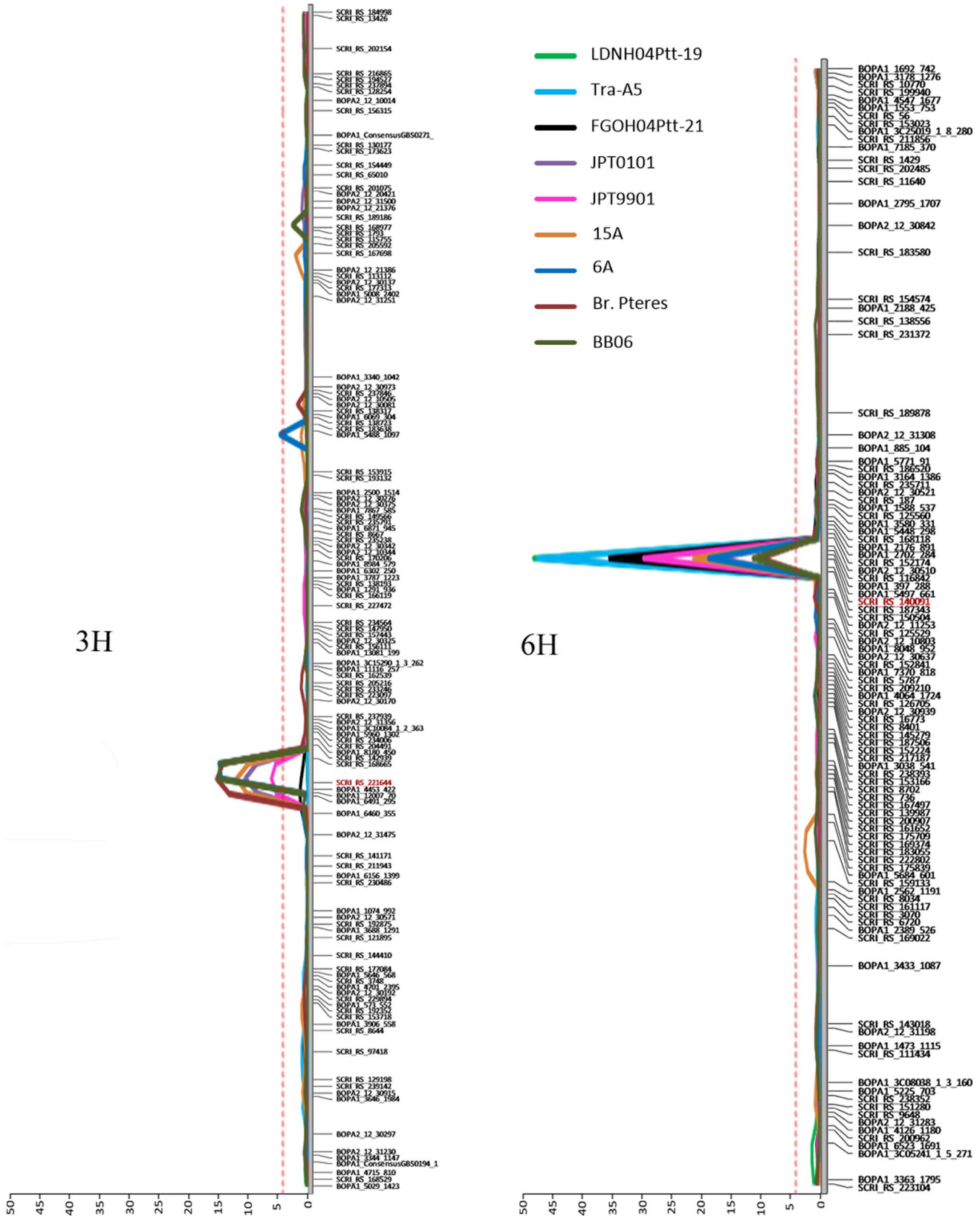

Fig. 3 QTL analysis of resistance in the CT RIL population against a global collection of *P. teres* f. *teres* isolates. Chromosomes 3H and 6H of barley are shown with markers to the right of the corresponding QTL composite interval mapping curve. LOD scales (0–50) are shown on the *x*-axis. The *dotted line* indicates the LOD threshold of 4.0 ($P = 0.01$). The most significant marker for each QTL is shown in red

been defined as a necrotrophic pathogen (Liu et al. 2011) and we have shown that *P. teres* f. *teres* produces NEs that lead to NE-triggered susceptibility (NETS) (Liu et al. 2015). In this study, however, we have identified two single gene sources of dominant resistance, one of which (6H) was effective against all of the isolates tested and the other (3H) showing differential reactions across the set of isolates that we used, as well as showing resistance being conferred by different barley parental lines. We speculate that the level of resistance conferred by both the 6H and 3H loci is an early response in the host-pathogen interaction that limits either penetration altogether or any proliferation immediately after penetration. Further investigation including microscopy studies is necessary to understand the temporal and spatial occurrence of this resistance mechanism. Additionally, characterization of the mode of pathogen recognition includes the identification of this resistance gene and the identification of the pathogen effector triggering this high level of resistance will be necessary to fully understand this host-pathogen interaction.

Author contribution statement VMK, TLF designed the experiments. VMK, SC performed the experiments. VMK, JDF, TLF, RSB, JKR analyzed the data. VMK, TLF, JKR wrote the paper.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflicts of interest for this article.

Ethical standards All experiments performed complied with the ethical standards of the USDA-ARS and North Dakota State University.

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